by the naked eye. The mirror image rule is obeyed by these compounds. The ratio of emission at 7100 Å to that at 6500 Å goes as the ratio of absorption at 5900 Å to that at 6500 Å. Also, the energy spacings between the emission peaks (1400–1500 cm^{-1}) are approximately equal to the energy spacings between the absorption maxima.

We are continuing the investigation of these new materials. We are hopeful that some of these new derivatives, tetra(*o*-bromophenyl)porphin in particular, will exhibit easily detectable phosphorescence as the free bases since the fluorescence is appreciably quenched. As far as we can determine, there has been only one positive report on the observation of phosphorescence of metal-free porphyrinic materials.[18] We have begun a more quantitative study of the emission properties and a broad matrix isolation study of the infrared and electronic spectra of these porphyrins.

(18) K. E. Riekhoff and E. M. Voigt, "Molecular Luminescence," E. C. Lim, Ed., W. A. Benjamin, New York, N. Y., 1969, p 295ff.

Nucleoside *S*-Alkyl Phosphorothioates. VII.[1] A Fragment from the Nonsense Strand of a Modified *S*-Peptide "Gene"

Mohindar S. Poonian, Eugene F. Nowoswiat, and Alexander L. Nussbaum*

Contribution from the Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received October 21, 1971*

Abstract: The chemical synthesis of a dodecadeoxyribonucleotide of sequence AAGACAGCATAT is described. Using the *S*-ethyl group for protection of the 5′ terminus of an anchoring dinucleotide, fragment condensation consisted of three sequential dimer additions, followed by a tetramer addition. This sequence constitutes a portion of the nonsense strand of a double-stranded polymer coding for a modified *S*-peptide of pancreatic ribonuclease A.

Previous papers from this laboratory[1,2] have described synthetic work on deoxyribonucleotide oligomers constituting fragments of a projected double-stranded array of DNA coding for a derivative of the *S*-peptide from ribonuclease A. The present work is a continuation in part, describing the synthesis of a dodecanucleotide run in the nonsense chain. It will be seen that the oligomer in question (upper line) overlaps, by antiparallel base pairing in the sense of Watson and Crick, a previously described[2] tridecadeoxyribonucleotide

5′--A-A-G-A-C-A-G-C-A-T-A-T--3′
3′--C-G-T-A-T-A-C-C-T-A-A-T-T--5′

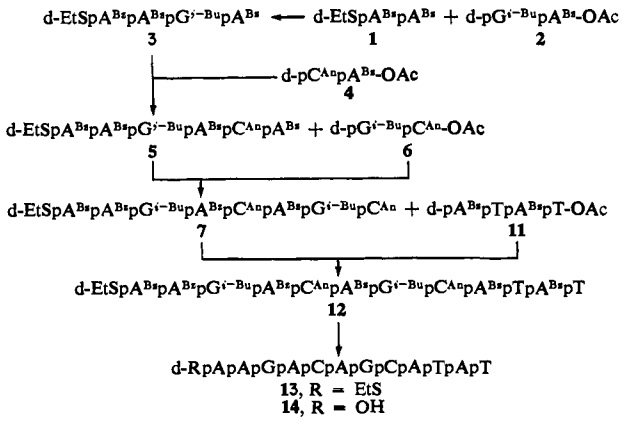
(bottom line) which is to form a part of the complementary chain (the sense strand) of the "gene."

Scheme I summarizes the synthetic scheme. Synthesis proceeded in the usual 5′ to 3′ direction, the 5′ terminus being protected by the *S*-ethyl phosphorothioate function throughout. The starting dimer **1**[1] was condensed with **2**[1] to give tetramer **3**, a species which, upon removal of protecting groups, is isomeric to the AAAG previously described.[3] Purification of the blocked tetramer required two successive fractionations with DEAE-cellulose, the final yield being 17.3%.

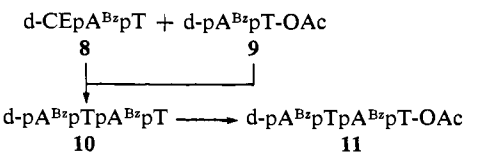
A second condensation with the dimer d-$pC^{An}pA^{Bz}$-OAc[4] (**4**) gave the hexamer **5** in similar yields as before (17.4%). Further condensation with considerable excess (23-fold) of d-$pG^{i\text{-}Bu}pC^{An}$[5] (**6**) gave a rather higher yield (48%) of the octamer **5** although the latter was not entirely pure. The material could, however, be used in the next step.

Synthesis of the 3′-terminal tetramer, shown in Scheme II, was carried out in the conventional manner.

Scheme I. Dodecamer Synthesis



Scheme II. Synthesis of the 3′-Terminal Tetramer



Dimers d-CEpA^{Bz}pT (**8**) and d-pA^{Bz}pT-OAc[6] (**9**) gave the desired tetramer **10**, which was acetylated to **11** for protection of the 3′ terminus.

The final condensation employed a 50-fold excess of the incoming (*i.e.*, phosphate donor for the newly

(1) Paper VI in this series: E. Heimer, M. Ahmad, A. Ramel, and A. L. Nussbaum, *J. Amer. Chem. Soc.*, 94, 1707 (1972).
(2) A. F. Cook, E. P. Heimer, M. J. Holman, D. T. Maichuk, and A. L. Nussbaum, *ibid.*, 94, 1334 (1972).
(3) T. M. Jacob, S. A. Narang, and H. G. Khorana, *ibid.*, 89, 2177 (1967).
(4) A. Kumar and H. G. Khorana, *ibid.*, 91, 2743 (1969). For nomenclature, see earlier references.[1,2]
(5) H. Schaller and H. G. Khorana, *ibid.*, 85, 3841 (1963).
(6) S. A. Narang, S. K. Dheer, and J. J. Michniewicz, *ibid.*, 90, 2702 (1968).

Table I. Description of Peaks from Column Chromatography

Figure	Peak A	Peak B	Peak C	Peak D	Peak E
1	d-EtSpA^{Bz}pA^{Bz}	d-pG$^{i\text{-}Bu}$pA^{Bz}	d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}		
2	MSA[a]	Unknown	d-pC^{An}pA^{Bz} and d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}	Symmetrical pyrophosphate of d-pC^{An}pA^{Bz}	d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}-pC^{An}pA^{Bz}
3	MSA[a]	d-pG$^{i\text{-}Bu}$pC^{An}	Symmetrical pyrophosphate of d-pG$^{i\text{-}Bu}$pC^{An}	d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}-pC^{An}pA^{Bz}	d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}-pC^{An}pA^{Bz}pG$^{i\text{-}Bu}$pC^{An}
4	MSA[a]	Unknown	d-pA^{Bz}pT	Symmetrical pyrophosphate of d-pA^{Bz}pT	d-pA^{Bz}pTpA^{Bz}pT
5	d-pA^{Bz}pTpA^{Bz}pT	d-EtS octamer	Symmetrical pyrophosphate of tetramer	d-EtS dodecamer	

[a] Mesitylenesulfonic acid.

Table II. Nucleotide Compositions in Mole %[a] (Theory)

Oligonucleotide	dpT	dpC	dpG	dpA
d-EtS-pApApGpA			26.5 (25)	73.5 (75)
d-EtS-pApApGpApCpA		18.1 (16.6)	18.2 (16.6)	63.7 (66.7)
d-EtS-pApApGpApCpApGpC		24.8 (25)	25.3 (25)	49.8 (50)
d-pApTpApT	50.1 (50)			49.9 (50)
d-EtS-pApApGpApCpApGpCpApTpApT	18.0 (16.6)	15.9 (16.6)	14.5 (16.6)	51.5 (50.0)
d-pApApGpApCpApGpCpApTpApT	17.8 (16.6)	17.0 (16.6)	15.8 (16.6)	49.2 (50.0)

[a] Determination by high-pressure liquid chromatography after deacylation and snake venom diesterase digestion.

Table III. Uv Characteristics of Protected Oligonucleotides

	λ_{max}, nm	λ_{min}, nm	Ratio 260/280	Ratio 300/280
d-EtS-pA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}	279 (257 sh)	230	0.76	0.40
d-EtS-pA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}pC^{An}pA^{Bz}	281 (259 sh)	230	0.75	0.57
d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}pC^{An}pA^{Bz}pG$^{i\text{-}Bu}$pC^{An}	281 (260 sh)	232	0.81	0.63
d-pA^{Bz}pTpA^{Bz}pT	277 (261 sh)	235	0.77	0.33
d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}pC^{An}pA^{Bz}pG$^{i\text{-}Bu}$pC^{An}-pA^{Bz}pTpA^{Bz}pT	278 (260 sh)	237	0.85	0.50

formed phosphodiester bond) fragment, **11**, over the accepting octamer (**7**, Scheme I). The protected dodecamer **12** was obtained in acceptable yield and deacylated with ammonia. The resulting material was purified by ion-exchange chromatography in the presence of urea[7] and characterized, in addition to the usual criteria (see General Methods), by its molecular weight (determined in the ultracentrifuge by a sedimentation equilibrium[2] method). Finally, removal of the 5′-terminal *S*-ethyl substituent by oxidative hydrolysis[8] gave the desired dodecamer **14**. Structure, as usual, was confirmed by monomer composition, molecular weight, and specific labeling of the 5′ terminus; the primary phosphate was removed with phosphomonoesterase and replaced with ^{32}P *via* polynucleotide kinase[9] catalysis. After hydrolysis to the constituent mononucleotides, incorporation of ^{32}P into deoxyadenylic acid only was observed, as required by the postulated structure.

Further work in extending both "sense" and "nonsense" strands is in progress and will be duly communicated.

Experimental Section

Materials. Reagent grade pyridine (Fisher Scientific Company) was first distilled over chlorosulfonic acid and subsequently over potassium hydroxide and was stored over Molecular Sieves. This sample of dry pyridine was used in all condensation reactions and for solubilizing nucleotidic materials before precipitation. For routine concentrations of the column effluents containing triethylammonium bicarbonate (TEAB), undistilled reagent grade pyridine was added. Mesitylenesulfonyl chloride (Aldrich Chemicals) was recrystallized twice from *n*-pentane and stored in a desiccator over P_2O_5 for subsequent use. Reagent grade triethylamine (Matheson Coleman and Bell) was used without further purification for preparation of triethylammonium bicarbonate buffers.

Table IV. Paper Chromatographic R_f Values of Oligodeoxyribonucleotides

Oligomers	Solvent system A	B	C	D
d-EtSpApApGpA	0.32	0.47	0.36	0.49
d-EtSpApApGpApCpA		0.15	0.18	0.34
d-EtSpApApGpApCpApGpC		0.07	0.06	0.24
d-pApTpTpApT		0.23	0.32	0.15
d-EtSpApApGpApCpApGpCpApTpApT		0.50[a]		
d-pApApGpApCpApGpCpApTpApT		0.64[b]		

[a] R_f with respect to EtS octamer. [b] R_f with respect to EtS dodecamer.

General Methods. A typical condensation reaction between the 3′-hydroxyl functionality of an appropriately protected oligomer (or a monomer) and the 5′-phosphate group of the incoming, suitably protected, nucleotide fragment involved use of an excess of the latter. Mesitylenesulfonyl chloride (0.7 equiv/phosphate charge) was used as a condensing agent. Distilled dry pyridine was used as a reaction medium. Anhydrous reaction conditions were achieved by repeated evaporation of the reactant nucleotides with dry pyridine. Finally, the syrupy residue was dissolved in anhydrous pyridine and the volume of the reaction solution brought down by evaporation so that the concentration of the reactants was

(7) R. V. Tomlinson and G. M. Tener, *Biochemistry*, **2**, 697 (1963).
(8) A. L. Nussbaum and R. Tiberi, *J. Amer. Chem. Soc.*, 87, 2513 (1965).
(9) B. Weiss, T. R. Live, and C. C. Richardson, *J. Biol. Chem.*, **243**, 4530 (1968).

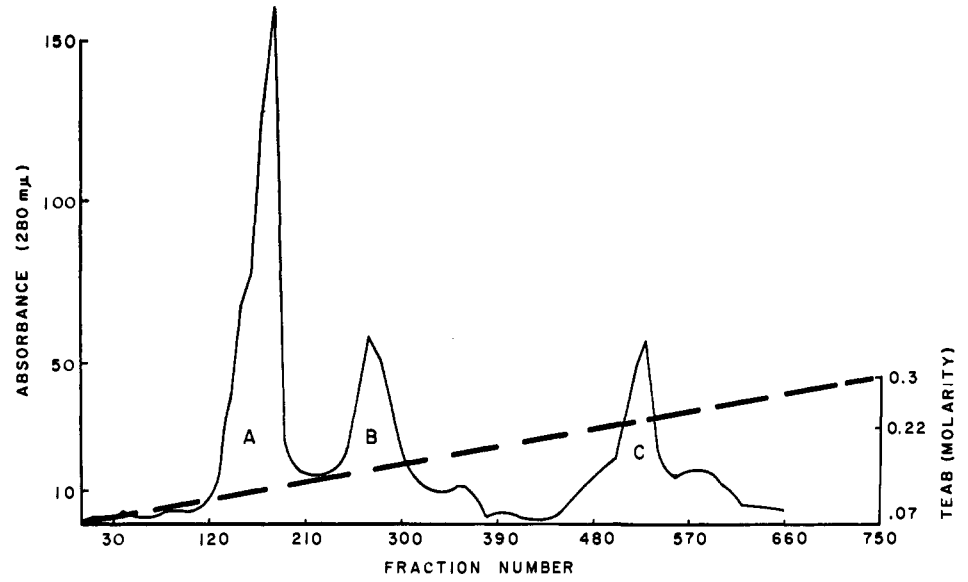


Figure 1. DEAE-cellulose (DE-23) (bicarbonate) chromatography of the reaction between d-EtS-pA^{Bz}pA^{Bz} and d-pG$^{i\text{-}Bu}$pA^{Bz}-OAc. A triethylammonium bicarbonate (pH 7.0) linear gradient 0.07–0.32 *M* (8 l. each vessel) was applied on a column size of 5 × 75 cm. Fractions of 20 ml were collected.

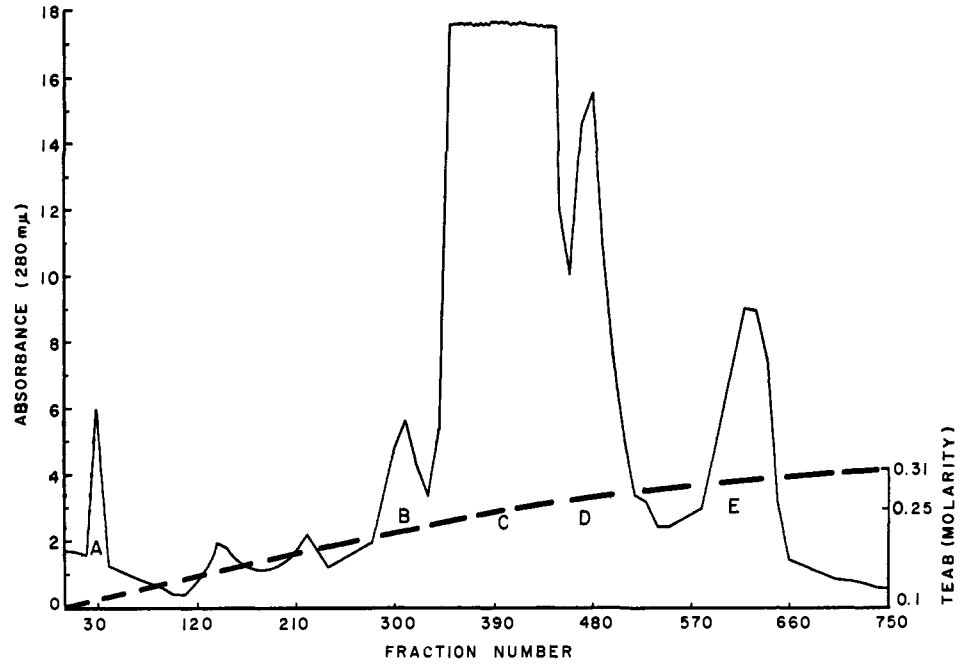


Figure 2. DEAE-cellulose (DE-23, bicarbonate) chromatography of the condensation reaction between a tetramer d-EtS-pA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz} and the incoming dimer d-pC^{An}pA^{Bz}-OAc. A convex gradient (0.1–0.35 *M* triethylammonium bicarbonate, pH 7.0, 8 l. of mixing vessel) was applied on a 5 × 71 cm column. Fractions of 20 ml were collected.

maximized without making the solution too viscous. A calculated amount of the condensing agent was then added and quickly dissolved by shaking the flask. The reaction was allowed to proceed in the dark at room temperature on a wrist action shaker for 2.5–3 hr. At the end of the reaction, the contents were cooled in an ice bath and quenched by adding a cold pyridine solution of *N,N*-diisopropylethylamine (equimolar to condensing agent) and cold water (equal to the reaction volume). The solution so obtained was kept at 4° overnight and then treated with 1 *M* NaOH on ice for 15 min to hydrolyze selectively the 3′-*O*-acetyl substituent. The basic solution was neutralized with the addition of the pyridinium form of Dowex-50 resin. The solution was separated from the resin, appropriately diluted with water, and applied to a DEAE-cellulose (bicarbonate) column (at 4°) which had previously been equilibrated with the dilute TEAB buffer (pH 7.4). The material was eluted by applying a suitable gradient (convex or linear) of TEAB (pH 7.4). The emergence of the nucleotidic materials was monitored by uv spectroscopy. The peaks were identified on the basis of uv spectrum, paper and thin-layer chromatography[10] (on deacylated materials), sulfur test,[11] and the nucleotide analysis after digestion with venom phosphodiesterase. At times it was found necessary to rechromatograph the product peak using shallower gradients. The homogeneous portions of the peaks were pooled and concentrated with pyridine (bath temperature never exceeding 30°) under vacuum on a flash evaporator. Most of the TEAB

(10) A new thin-layer chromatographic system was found to be useful in analysis of oligomers up to dodecamer. Silica gel thin-layer plates were developed in ethanol–methanol–0.1 *M* SDS (sodium dodecyl sulfate) (5:2:3 v/v) over a period of 7 hr. Details and variations of this system will be published elsewhere.

(11) T. Wieland and R. Lambert, *Chem. Ber.*, 89, 2976 (1956).

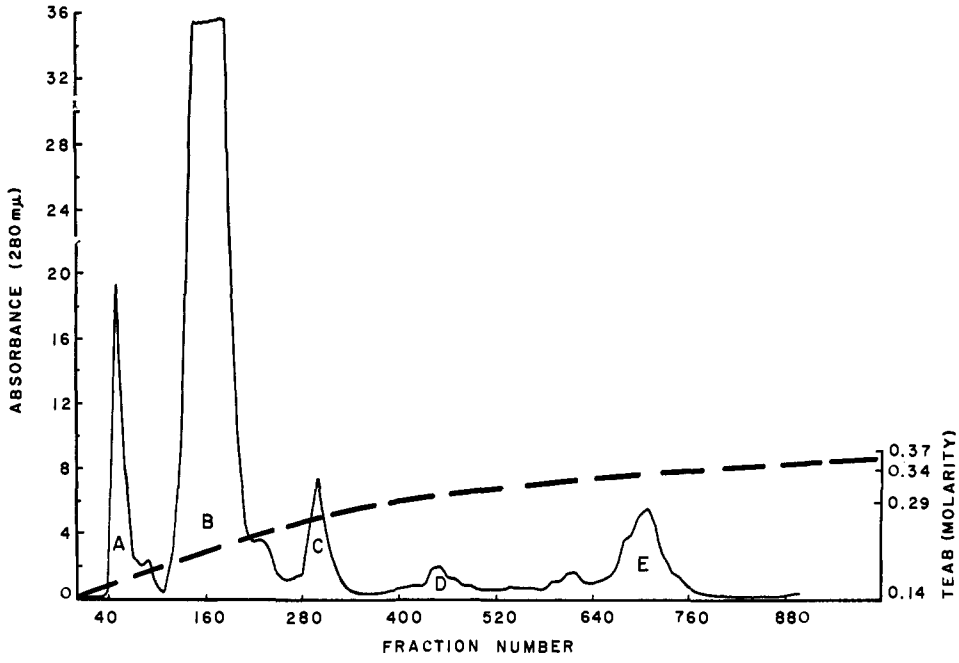


Figure 3. DEAE-cellulose (DE-23, bicarbonate) chromatography of the products of the reaction between d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}pC^{An}-pA^{Bz} and pG$^{i\text{-}Bu}$pC^{An}-OAc. A convex gradient of 0.14–0.38 *M* triethylammonium bicarbonate buffer (pH 7.0) with 8 l. of mixing vessel was applied on a 3.5 × 65 cm column. Volume of each fraction collected was 15 ml.

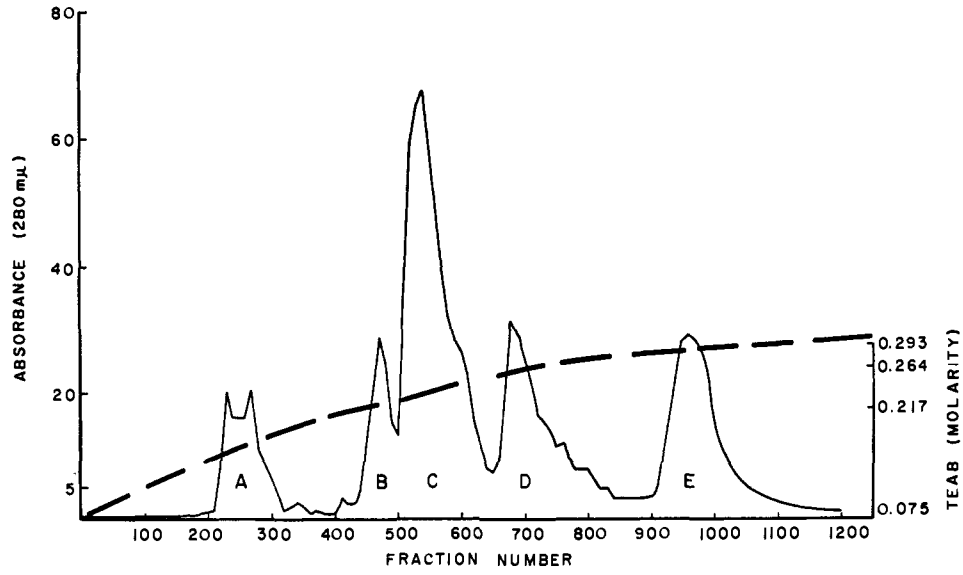


Figure 4. DEAE-cellulose (DE-23, bicarbonate) chromatography of the products of the condensation between d-CEpA^{Bz}pT and d-pA^{Bz}pT-OAc. A convex gradient of triethylammonium bicarbonate (0.075–0.3 *M*, pH 7.0) with 7 l. of mixing vessel was applied. Fractions of 20 ml were collected.

buffer thus having been removed, the residue was dissolved in dry pyridine and the product precipitated by dropping the pyridine solution gradually into anhydrous ether. The precipitate was filtered or centrifuged, washed, and dried under vacuum over P_2O_5. This material was then used for further condensations.

Column chromatography of the individual condensations is documented in the figures. A description of the individual peaks is contained in Table I. Moreover, composition for the new compounds is listed in Table II. Pertinent uv characteristics are compiled in Table III, and Table IV specifies some R_f values in the following systems: A, rapid paper chromatographic system,[12] acetonitrile–0.1 *M* ammonium acetate, 50:50 (v/v); B, 1-propanol–water–concentrated ammonia, 65:35:10 (v/v); C, ethanol–1 *M* ammonium acetate, 50:50 (v/v); D, 1-propanol–0.05 *M* ammonium acetate, 50:50 (v/v) (this system separates the larger oligomers satisfactorily in 20 hr).

Preparation of d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz} (3). The protected dimer d-EtS-pA^{Bz}pA^{Bz} (**1**, 3.04 g, 3 m*M*) was condensed with d-pG$^{i\text{-}Bu}$pA^{Bz}-OAc (**2**) (5.86 g, 6 m*M*) in the presence of mesitylenesulfonyl chloride (16.8 m*M*) in anhydrous pyridine (90 ml) for 2.5 hr. The reaction mixture was treated with cold water, and the 3′-*O*-acetyl protecting group was removed as discussed under General Methods. The material was then chromatographed on a DE-23 DEAE-cellulose (bicarbonate form) column (5 × 75 cm), using a linear gradient of triethylammonium bicarbonate (pH 7.4) between 0.07 and 0.32 *M* (8 l. each vessel). Identification of the several peaks (Figure 1) is given in Table I. From the tetramer peak, fractions 500–550 were pooled. Since this material had 10–15% contaminants, it was rechromatographed [DE-23, 3.5 × 69 cm, convex gradient of TEAB (pH 7.4) between 0.18 (8 l.) and

(12) T. F. Gabriel, *J. Chromatogr.*, **36**, 518 (1968).

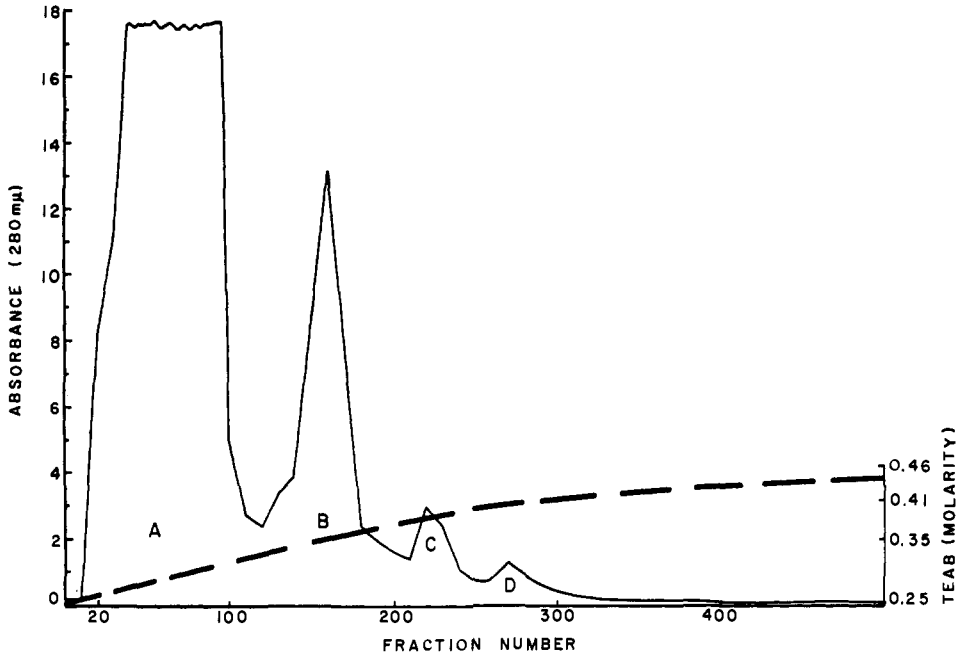


Figure 5. DEAE-cellulose (DE-23, bicarbonate) chromatography of the reaction between Et-S-pA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}pC^{An}pA^{Bz}pG$^{i\text{-}Bu}$pC^{An}-OH and the tetramer d-pA^{Bz}pTpA^{Bz}pT-OAc. A convex gradient of triethylammonium bicarbonate (pH 7.0) 0.25–0.5 *M* (4.5 l. of mixing vessel) was applied on a 2 × 90 cm column. Fractions of 15 ml were collected.

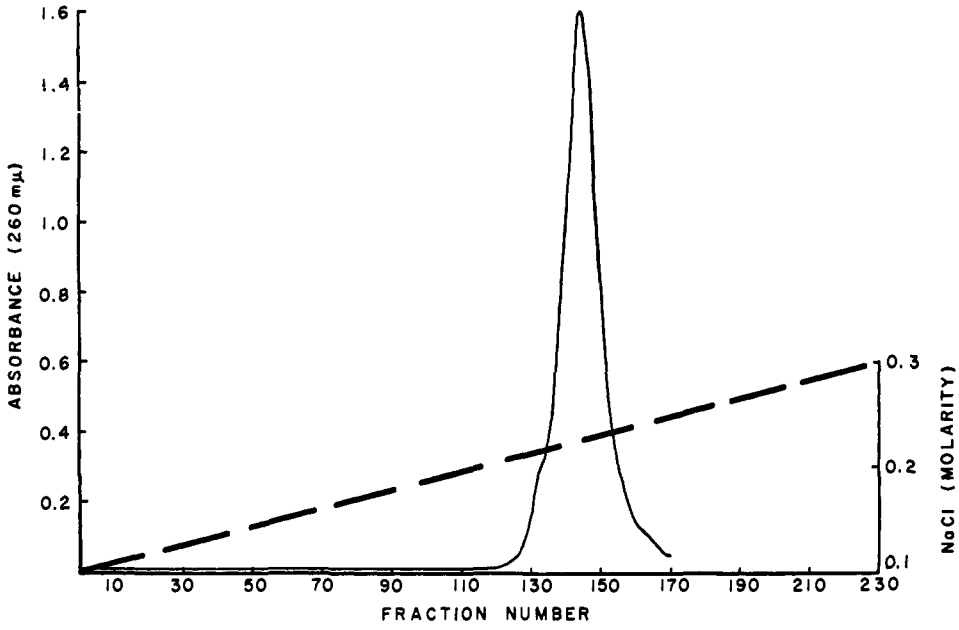


Figure 6. DE-52 chromatography of deacylated *S*-ethyl dodecamer in 7 *M* urea and 0.02 *M* Tris (pH 7.4). A linear gradient of NaCl, 0.1–0.3 *M* (400 ml each), was used and fractions of 3.5 ml collected. Appropriate fractions were pooled and desalted on G-15 Sephadex.

0.25 *M*]. The yield of the EtS tetramer was 35,026 OD units (0.52 m*M*, 17.3%).

Hexamer d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}pC^{An}pA^{Bz} (5). The above sample of tetramer d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz} (**3**, 0.5 m*M*) was treated with a tenfold excess (5 m*M*) of the protected dimer block d-pC^{An}pA^{Bz}-OAc, **4**, in the presence of mesitylenesulfonyl chloride (2.6 g, 11.9 mmol) in anhydrous pyridine (10 ml) under the conditions described before. The reaction was treated with water and, after selective removal of 3′-protecting group, it was chromatographed on a DEAE-cellulose column (5 × 71 cm) using a convex gradient (8 l. of mixing vessel) of 0.1 *M* TEAB–0.35 *M* TEAB (pH 7.4) (see Figure 2). The hexamer peak (Table I) from fractions 580–650 was found to be homogeneous and consisted of 8236 OD units (87 μmol, 17.4% yield).

Octamer d-EtSpA^{Bz}pA^{Bz}pG$^{i\text{-}Bu}$pA^{Bz}pC^{An}pA^{Bz}pG$^{i\text{-}Bu}$pC^{An} (7). The entire quantity of the above hexamer (**5**, 87 μmol) was utilized in condensation with d-pG$^{i\text{-}Bu}$pC^{An}-OAc (**6**, 1.97 g, 2.0 m*M*, 23-fold excess). The reaction was carried out in anhydrous pyridine (8 ml) in the presence of mesitylenesulfonyl chloride (1 g, 4.58 m*M*) for 3 hr at room temperature. The octamer peak (Table I, Figure 3) was pooled between fractions 670 and 770 and the pool was found by pc to be greater than 90% pure in the desired product. The yield of 5460 OD units (41.8 μmol, 48%) was thus realized. No attempt was made to purify this material further.

Synthesis of d-pA^{Bz}pTpA^{Bz}pT-OAc (11). d-CEpA^{Bz}pT-OH (**8**, 4.8 m*M*) and d-pA^{Bz}pT-OAc (**9**, 8.47 m*M*) were condensed together in the presence of mesitylenesulfonyl chloride (24.4 m*M*) in a total reaction volume of 60 ml of dry pyridine solution. A reaction time of 2.5 hr at room temperature in the dark was allowed. After quenching and acetate hydrolysis, the products were chromatographed on a DEAE-cellulose column (7 × 88 cm) using a convex gradient (7 l. of mixing vessel) of 0.075 *M* to 0.3 *M* TEAB (pH 7.4).

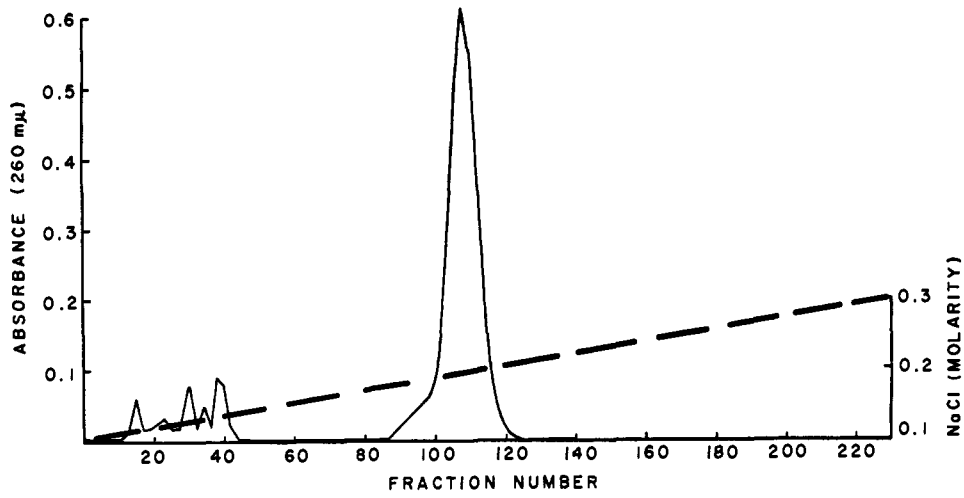


Figure 7. DE-52 chromatography of completely deprotected dodecamer in 7 *M* urea and 0.02 *M* Tris (pH 7.4). The sample of deacylated *S*-ethyl dodecamer (24.0 OD) was treated with a mixture of KI and I_2 in phosphate buffer (see text) for 1 hr at room temperature. Unreacted iodine was removed by repeated extraction with CCl_4. The material was then applied on a column in 7 *M* urea, 0.02 *M* Tris (pH 8.0) and a gradient of NaCl (0.1–0.3 *M*) was (7 *M* urea and 0.02 *M* Tris, pH 7.4) then applied. Fractions of 3.5 ml were collected.

The chromatographic pattern is presented in Figure 4 and characterization of peaks in Table I. The desired tetramer material was pooled between fractions 920 and 1075 (51,315 OD units, 1.06 m*M*, 22% yield). The effluent was concentrated with dry pyridine and the tetramer **10** precipitated into dry ether. The purified and dry precipitate was then acetylated with acetic anhydride and dry pyridine. Excess of acetic anhydride was hydrolyzed by addition of cold water to a cooled reaction mixture. After the usual procedure of concentration with dry pyridine, the acetylated tetramer d-pA^{Bz}-$pTpA^{Bz}pT$-OAc (**11**) was precipitated with dry ether. The dried precipitate was then used in condensation with the alkylthio octamer **7**.

Dodecamer d-EtS$pA^{Bz}pA^{Bz}pG^{i\text{-}Bu}pA^{Bz}pC^{An}pA^{Bz}pG^{i\text{-}Bu}pC^{An}pA^{Bz}pTpA^{Bz}pT$-OH (12). A sample of octamer d-EtS$pA^{Bz}pA^{Bz}pG^{i\text{-}Bu}pA^{Bz}pC^{An}pA^{Bz}pG^{i\text{-}Bu}pC^{An}$ (**7**, 20 μmol) was condensed with pA^{Bz}-$pTpA^{Bz}pT$-OAc (**11**, 1.06 m*M*, 50-fold excess) in the presence of mesitylenesulfonyl chloride (3.82 m*M*) in anhydrous pyridine medium (total reaction volume, 4.5 ml) for 3 hr at room temperature with vigorous shaking. After the usual procedure of hydrolysis of the 3′-*O*-acetyl substituent, the material was chromatographed on DE-23 (bicarbonate) column (2 × 90 cm) using a convex gradient of TEAB (pH 7.4), 0.25–0.5 *M* (mixing vessel, 4.5 l.). The chromatographic pattern is depicted in Figure 5 and the identification of various peaks in Table I. Based on paper and thin-layer chromatographic analysis on deacylated material, the EtS dodecamer peak was pooled between fractions 250 and 400. A total of 648 OD units was thus obtained representing a yield of 3.6 μmol (18%). Further purification of the EtS dodecamer product was achieved by chromatographing the deacylated material on Tener columns[7] as described below.

Urea Column Chromatography on the Deacylated EtS Dodecamer. A sample of protected EtS dodecamer (603 OD_{278} units) was treated with concentrated ammonia at room temperature for 3 days. The reaction mixture was concentrated and dissolved in water. The total material at this stage corresponded to 520 OD units at 258 mμ (of this, some 460 OD_{258} units are calculated to be nucleotidic; the rest result from protecting groups). Out of this, 234 OD_{258} units were chromatographed on a DEAE-cellulose (DE-52)c olumn (0.9 × 88 cm) in the presence of 7 *M* urea and 0.02 *M* Tris (pH 7.5). A linear gradient of NaCl (0.1–0.4 *M*, 400 ml each vessel) was used. The main peak of the nucelotidic material was divided into two portions on the basis of the peak homogeneity. Both portions I and II were desalted on Sephadex G-15 (2.5 × 80 cm) and subjected to rechromatography on a Tener column[7] (DE-52) under the conditions described above, except that the NaCl gradient used was 0.1–0.3 *M*. From portion II were obtained 76 OD_{258} units (pattern Figure 6) of EtS dodecamer which was characterized by nucleotide analysis, molecular weight determination (3700, 3792 theory), and tlc analysis. The first portion provided 60 OD_{258} units of EtS dodecamer of slightly lower purity. The total recovery of EtS dodecamer (136 OD_{258} units) therefore corresponds to 65%.

Removal of *S*-Ethyl Substituent from EtS Dodecamer 13. The EtS dodecamer (24 OD units) was treated with a solution which contained water (0.15 ml), 0.4 *M* PO_4 buffer (pH 7.4, 0.15 ml), 0.1 *M* I_2, and 0.2 *M* KI (0.3 ml)[13] for 1 hr at room temperature. Unreacted iodine was extracted with carbon tetrachloride (five times with 1 ml). After appropriate concentration, the material was chromatographed on a Tener column (conditions same as above). The pattern is given in Figure 7. Fractions 100–120 were pooled, desalted, and concentrated to a suitable volume. The total material recovered at this stage was 20.0 OD units. This material was analytically pure and had a molecular weight of 3820 (3748 theory). Approximately 1 OD unit of this material was treated with bacterial alkaline phosphatase to hydrolyze the 5′-phosphate and the 5′ terminus was then labeled with [^{32}P]phosphate under the action of polynucleotide kinase. A venom phosphodiesterase digest of the labeled material was resolved by high-pressure liquid chromatography to reveal the presence of radioactivity only under deoxyadenosine 5′-phosphate peak, confirming thereby the integrity of the 5′ terminus of the dodecamer fragment.

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(13) Recent studies (Cook and colleagues of this laboratory) have shown that these conditions are more satisfactory for the removal of *S*-alkyl blocking groups than those previously employed; among other factors, the reaction is cleaner and more rapid.